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1. Current literature highlights

1.1. Bicyclic peptides from cysteine-rich phage libraries

Cyclic peptides can bind to protein surfaces with high affinity. There are a number of naturally occurring cyclic peptides that have drug-like properties and that are approved for human therapeutic use. In addition cyclic peptides have considerable use as pharmacological tools. The high affinity that cyclic molecules confer is in part due to reduced entropic loss on binding, often enhanced by a cyclic structure that holds the binding epitope in an active conformation.

Because of this precedent for activity, there is considerable interest in seeking cyclic ligands from chemical and phage libraries. A recent study has investigated how phage-encoded combinatorial peptide libraries can be spontaneously cyclised through cysteine side chains by air oxidation [1].

Monocyclic peptides containing two cysteine groups are often isolated from phage libraries, and occasionally show high affinity for protein targets. Including four cysteine residues will potentially generate bicyclic systems with additional diversity conferred by alternative cyclisation topologies. This approach is an attractive alternative to cyclising phage-derived peptides with tris-(bromomethyl)benzene (TBMB) prior to affinity selection screening. Although TBMB-cyclised phage libraries have generated potent ligands for plasma kallikrein, cathepsin G and the serine protease, urokinase-type plasminogen activator (uPA), this type of cyclisation is a technically challenging step in the library preparation.

Thus, this recent study generates two types of bicyclised phage library: a 6×6 library with the sequence CX_6CX_6C, in which the X positions contain all amino acids including cysteine, and a 4×4 library with the sequence XCX_4CX_4CX. The random amino acid positions were encoded by 'NNK' codons resulting in a 1 in 32 chance of a random cysteine in each of the X positions. The former 6×6 library (size: 4×10^9 peptides) was produced, cyclised by oxidation and then screened by affinity selection against the model target, streptavidin. It has previously been shown that the 'HPQ' consensus sequence is often found in peptides that bind to streptavidin, and indeed was found in many of the most enriched sequences in this work. The second phage library (library size: 4×10^8) was screened against uPA, and a number of consensus sequences were identified. Several bicyclic peptides were subsequently synthesised and two preferred compounds, UK501 and UK504, had measured Ki values of 4.3 and 7.7 μM respectively.

Overall, it was assumed that bicyclic libraries generated by oxidation had high topological diversity as the fourth cysteine can occur in any one of the randomized amino acid positions. This enhances their diversity relative to the TBMB-cyclised peptides, where the cyclisation positions were defined in advance. That these bicyclic peptides can generate active binders was demonstrated by the discovery of uPA binders after only two rounds of iterative panning. Finally, a crystal structure showed the bicyclic peptide UK504 bound to uPA, possessing a rigid structure and fully occupying the substrate-binding site of the target.

2. A summary of the papers in this month's issue

2.1. Polymer supported synthesis

Protein–protein interactions (PPIs) mediate cellular pathways and have been implicated in numerous aberrant conditions. α-Helix mimetics – small molecules that reproduce the spatial projection of key residues from an α-helix involved in a PPI – are attractive generic templates for development of screening libraries. However library syntheses of α-helix mimetics with diverse functionality are not well established. A recent publication describes automated, microwave-assisted solid phase synthesis based on one such α-helix mimetic scaffold; an *N*-alkylated oligobenzamide [2].

2.2. Solution-phase synthesis

An efficient one-pot tandem method for the synthesis of pyridazino [4,5-*b*] [1,4]thiazine-diones via the Smiles rearrangement has been developed. A number of products were obtained in high yields without any by-products. This transition metal-free process is an environmentally friendly, economical, and efficient method for preparation of mixture-based heterocyclic libraries [3].

The novel syntheses of 12-membered macrocyclic templates and a library of 4000 macrocyclic analogues have been reported. The key macrocyclisation step was performed at up to 100 g scale without resorting to syringe pumps, flow reactors or large volumes of solvent. An interesting observation of considerably different permeability was made on diastereomeric analogues due to differences in intramolecular hydrogen bond interactions [4].

Diverse hydrazones have been oxidised to the corresponding diazoalkanes using sodium hypochlorite in the presence of catalytic amounts of TEMPO (2,2,6,6-tetramethylpiperidinyloxy).

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A library of diverse benzhydryl esters and analogues has been prepared from diazoalkanes obtained by this procedure [5].

An efficient regio- and chemoselective method for the synthesis of novel 3-amino-2′-oxospiro[benzo[*c*]pyrano [3,2-a] phenazine-1,3′-indoline]-2-carbonitrile/carboxylate derivatives has been developed via the one-pot, four-component domino coupling of 2-hydroxynaphthalene-1,4-dione, benzene-1,2-diamines, isatins, and malononitrile/cyanoacetic ester. This reaction proceeded in the presence of DABCO under reflux conditions in excellent yields, and is highlighted by high atom-economy and efficiency in producing five new bonds (two C–N, two C–C, and one C–O). The approach was employed in developing a simple and versatile procedure for the combinatorial synthesis of a spiro-substituted pyranophenazine-indoline library for biological screening [6].

An efficient route to novel 4,7-dihydro-1*H*-pyrrolo[2,3-*b*]pyridines with a tetrahedral fragment incorporated into position-4 has been described. The route uses a three-component reaction of *N*-substituted 5-amino-3-cyanopyrroles, various carbonyl and active methylene compounds. The reactions were carried out under mild conditions using ethanol, acetic acid or 1,4-dioxane as solvent and can be applied to the construction of 4-spiro-fused dihydro-1H-pyrrolo[2,3-b]pyridine libraries [7].

A small library of compounds has been prepared by a combination of toluene dioxygenase (TDO)-catalysed enzymatic dihydroxylation and copper(I)-catalysed Hüisgen cycloaddition. Some compounds were obtained by coupling an alkyne and a conduritol derivative, while more complex structures were obtained by a double Hüisgen reaction of a dialkyne and two molecules of the cyclitol. The compounds were fully characterised and subjected to preliminary biological screening [8].

2.3. Scaffolds and synthons for combinatorial libraries

A convenient microwave-assisted one-pot sequential synthesis has provided access to novel pyridopyrimidin-4-(3*H*)-ones in good to excellent yields. Anthranilic acid, 2- and 4-aminonicotinic acids, and 3-aminoisonicotinic acid were quantitatively converted into the analogous amidinoesters which undergo rapid cyclisation in the presence of an amine. This approach represents a reliable and simple method which can be applied to various ring systems and amines in order to generate libraries of chemical scaffolds [9].

2.4. Solid-phase supported reagents

Electrochemically deposited palladium nanoparticles on nafion–graphene support have shown excellent catalytic activity for Suzuki coupling reactions. The catalyst was characterised by SEM, TEM, EDAX, XRD, and TGA, with the particle size of palladium nanoparticles (Pd NPs) determined from TEM in the range of 4–12 nm. The recyclability of the catalyst was examined and no significant loss of catalytic activity was noted for five consecutive cycles [10].

A highly efficient Amberlite IR-120H resin mediated nucleophilic substitution of the hydroxyl group of propargylic alcohols with a wide range of nucleophiles has been reported. The reactions were achieved under very mild conditions and in excellent yields [11].

2.5. Novel resins, linkers and techniques

Repeat proteins are found in almost all cellular systems, where they are involved in diverse molecular recognition processes. Recent studies have suggested that *de novo* designed repeat proteins may serve as universal binders, and might potentially be used as practical alternative to antibodies. A novel chemical methodology for producing small libraries of repeat proteins, and screening ligand binding in parallel has been described. Novel, dynamic combinatorial libraries were designed and prepared, and it was shown that their equilibration can facilitate the formation of tetratricopeptide repeat proteins containing up to eight repeating units. Interestingly, equilibration of the library building blocks in the presence of the biologically relevant ligands, Hsp90 and Hsp70, induced their oligomerisation into forming more of the proteins with large recognition surfaces [12].

The synthesis of proteins by native chemical ligation greatly enhances the application of chemistry to complex molecules such as proteins. The essential building blocks for this approach traditionally have been peptide-thioester segments that are linked chemoselectively in consecutive reactions. By using peptide selenoesters instead of thioesters, the ligation rate can be significantly accelerated permitting couplings at difficult sites and potentially enabling new ligation strategies. To facilitate the routine synthesis of selenoester peptides, a general and straightforward procedure has been developed that generates a suitably functionalised resin from which the desired selenoester peptide can be readily synthesised. This simple approach uses readily available and cheap chemical agents and enables production of peptide selenoesters of excellent quality in short time and with high recovery [13].

2.6. Library applications

MDM2 and MDMX are oncoproteins that negatively regulate the activity and stability of the tumour suppressor protein p53. The inhibitors of protein–protein interactions (PPIs) of MDM2–p53 and MDMX–p53 are potential anticancer agents, and a recent study looked at novel approaches for identifying such inhibitors by affinity-based screening. A number of compounds from an in-house compound library, which were immobilised onto a chemical array, were screened for interaction with fluorescence-labelled MDM2 and MDMX proteins. A subsequent fluorescent polarisation assay identified several compounds that inhibited MDM2–p53 and MDMX–p53 interactions [14].

CCG-1423 is a novel inhibitor of Rho/MKL1/SRF-mediated gene transcription that inhibits invasion of PC-3 prostate cancer cells in a Matrigel model of metastasis. The design and synthesis of conformationally restricted analogues with improved selectivity for inhibiting invasion versus acute cytotoxicity were recently described. A recent study has described a survey of aromatic substitution with the goal of improving physicochemical parameters. Compound libraries were designed to explore diversity on the aromatic rings while modestly reducing lipophilicity, with the goal of overall increasing solubility [15].

A library of peptides and glycopeptides containing (4*R*)-hydroxy-L-proline (Hyp) residues have been designed with a view to providing stable polyproline II (PPII) helical molecules with antifreeze activity. A library of dodecapeptides containing contiguous Hyp residues or an Ala-Hyp-Ala tripeptide repeat sequence were synthesised with and without α-O-linked *N*-acetylgalactosamine and α-O-linked galactose-β-(1→3)-*N*-acetylgalactosamine appended to the peptide backbone. All (glyco)peptides synthesised displayed some ice recrystallisation inhibition activity with unglycosylated peptides containing the Ala-Hyp-Ala motif exhibiting the most potent inhibitory activity. Interestingly, although glycosylation is critical to the activity of native antifreeze glycoproteins that possess an Ala-Thr-Ala tripeptide repeat, this same structural modification is detrimental to the antifreeze activity of the Ala-Hyp-Ala repeat peptides studied here [16].

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